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V. Immunity in Insects[1]

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INTRODUCTION

Webster's Third New International Dictionary gives the following definition of immunity: "A condition of being able or the capacity to resist a particular disease, especially through preventing development of a pathogenic microorganism or by counteracting the effects of its products." This gives us ample room for discussion of aspects of immunity, in the broadest sense, not covered in detail in the excellent reviews by Briggs (7, 8) and Stephens (60). The insect pathologist soon finds that the protective mechanisms against microorganisms found in the blood are only part of the story of immunity, perhaps the lesser portion.

To relate the full story of the infectious process, one must begin with the means of invasion. When the agents employed by the invading organism are identified and isolated, one can then examine the counter agents produced by the insect. Since there is a paucity of such information, it is more a matter of educated guesswork than an exercise in relating scientific facts to write such a review. However, reflection on shortcomings is often rewarding through production of guidelines for future research.

INVASION

It is generally accepted that protozoa, bacteria, rickettsiae, and viruses gain entrance via the gut, although some bacteria (e.g., *Bacillus popilliae* Dutky) can gain entrance to the hemocoele through wounds (17).

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Fungi

Fungi invade the integument through wounds, via the trachea (33, 15), or via the gut. The precise mode of invasion by all of these routes is not known, but enzymes or mechanical pressure, or both, have been cited (36, 37).

The exoskeleton of the insect has been defined as a protein-chitin (*N*-acetyl glucosamine) mixture laid down by the hypodermal cells. This structure, hardened through a process of sclerotization of the protein, is coated with an epicuticle containing several substances but with wax as its major constituent. This rather formidable barrier is shed at more or less regular intervals by molting, a process that at times frustrates the attempted penetration of germinating fungal spores (Dutky, *personal communication*). In addition, this structure contains substances that inhibit fungi. For example, in some insects the cuticle contains unsaturated fatty acids, of medium-length chain, that are capable of inhibiting *Aspergillus flavus* Link (30). As a further example, Kawase (29) isolated 3,4-dihydroxybenzoic acid from silkworm exuviae. This compound is known to be antagonistic to fungi. Other substances that inhibit germination or growth of fungi may also be present in the cuticle of insects.

We are appallingly ignorant of the role played by the exoskeleton in discouraging growth and penetration of fungi; however, some of the habitats occupied by many insects are ideal for fungal

TABLE 1. *Enzymes produced by fungi pathogenic for insects**

Fungus	Lipases	Proteinases	Chitinase
Aspergillus flavus Link.	+	+	+
Beauveria bassiana (Balsamo) Vuillemin	+	+	+
Metarrhizium anisopliae (Metchnikoff) Sorokin	+	(−)	+
Cordyceps militaris (Linnaeus) Link.	+	+	+
Empusa muscae Cohn.	+	+	(−)

* Adapted from Benz (3).

development, and yet the insects survive, apparently repelling fungal invasion.

Table 1 shows the enzyme equipment possessed by some of the well-known fungal pathogens of insects. It should be noted that two of these pathogens do not possess a "full" set of enzymes. But it is also significant that we have no adequate proof that these enzymes have a specific role in invasion. All evidence for their implication is indirect.

Once fungi breach the exoskeleton, the insect is believed to be doomed, although some cellular resistance has been noted (22, 41, 6). This assumption that fungi invariably cause death after establishing in the hemocoele has never been validly proved. One can only conclude that much remains to be done in connection with invasion of insects by fungi.

Protozoa

Invasion by most entomogenous protozoa is an equal mystery, except for the Microsporidia. Workers in Europe and elsewhere have shown that spores of these organisms eject a hollow tube (called the polar filament) with sufficient force to penetrate the epithelial cell, and the sporoblast is injected into the host cell through this thin tube. It is almost certain from in vitro studies that the sporoblast must be injected into the cell to survive, and if by chance it is ejected into the lumen of the gut it perishes very quickly.

Viruses

In turning to the available explanations of invasion by viruses, one finds that the mode of action is still obscure. Researchers have initiated most virus studies at the nuclear level, ignoring the presence of resistance encountered by the virus from the time it is ingested until it arrives inside the cell. Aizawa (1), citing Day et al. (12) and Vago and Croissant (62), stated, "The polyhedra are dissolved by the alkaline gut juice, and the liberated particles penetrate through the gut epithelium and multiply in the cells of the blood and other tissues." In fact, Day et al. (12) observed, "We have no method for determining whether infection in the gut lumen occurs in the form of isolated rods or of rods within membranes, but the sequence illustrated represents our working hypothesis." Bird (5) suggested that, "Polyhedra are ingested by a larva and dissolved in the gut, liberating virus rods. The rods attach to midgut cells and release infectious subunits. These pass through the midgut epithelium, which in Lepidoptera are not susceptible to infection and enter a nucleus of a susceptible cell." This suggestion may or may not represent the actual sequence of events in virus invasion; however, Bird did not, and has not since, presented any evidence upon which such a hypothesis can be formed. There is no alternative but to return to the position of Day et al. (12) that evidence is lacking as to the viral invasion sequence.

Vago and Croissant (62) published an investigation of the action of gut juice from the silkworm [*Bombyx mori* (Linnaeus)] on the polyhedra of the specific nuclear polyhedrosis disease. Working with freshly extracted gut juice, they observed partial breakdown of polyhedra and partial release of intact virus particles in 100 to 130 sec after bathing polyhedra in undiluted gut juice from *B. mori*. After 60 min of exposure to gut juice, a large number of released rods were fragmented. Incidentally, X-ray studies showed that the fifth instar larva of *B. mori* requires 80 to 100 min to pass barium sulfate-coated mulberry leaves from mouth to anus (26). The chemical may actually accelerate the passage of food.

Vago and Croissant (62) tested virus released from polyhedra 10 and 60 min after treatment with gut juice. They applied these virus suspensions to eggs of *B. mori* (Bagdad strain) immediately before eclosion in full knowledge of the obligatory habit these larvae have of consuming at least part of the chorion immediately after eclosion. It is not clear whether they made any attempt at quantitation of virus; nevertheless, in 16 days virus exposed for 10 min to gut juice caused 42% mortality and virus exposed for 60 min caused only 12% mortality. Unfortunately, the authors did not include an equivalent amount of untreated polyhedra in a separate control; consequently, we cannot estimate the reduction of activity, if any, in the 10-min gut juice treatment experiment.

This experiment, preliminary as it is, lays a basis and guidelines for future work. Apparently, the virus particle is very quickly released in the gut and is not visibly damaged for some time,

suggesting that the particle itself is available as the infectious unit. Why, then, have we not evidence in Lepidoptera of the intact virus particle passing through the unsusceptible gut cells to the target tissues: fat cells, tracheal matrix, blood cells, and hypodermal tissue? Observations by competent investigators have not yielded any new information regarding the invasive particle (31, 12).

Bacteria

Although some bacteria (e.g., crystal-forming, sporeforming bacteria related to *B. thuringiensis* var. *thuringiensis* Berliner) contain toxic substances which can poison and kill insects, multiplication in the larval gut is essential in most bacterial infections. Therefore, conditions in the digestive tract may exert a great protective influence. The presence, in the gut, of antibacterial substances or suboptimal growth conditions due to, for example, unfavorable hydrogen ion concentrations may make development and multiplication of invading microbes virtually impossible.

Should the bacteria find conditions in the digestive tract to be favorable, they will germinate or multiply, or both. When conditions in the gut are suboptimal, they may barely maintain themselves, in numbers so low that they cannot be detected by conventional bacteriological techniques. Should a mechanical break occur in the gut wall, these bacteria are often capable of developing a full-blown septicemia that kills the host—thus, the name "potential pathogens," which Bucher (9) applied to these noninvasive organisms.

Let us consider the factors present in some insects that make conditions poor for development of bacteria (and possibly for other microorganisms as well).

Gut pH. Apparently the hydrogen ion concentration in the insect midgut is one of the most important factors governing the extent of multiplication of bacteria. In Lepidoptera, two general types exist. One group of insects maintains a *p*H above 9.0 throughout larval life, thus discouraging growth of bacteria by virtue of a high gut *p*H (56, 24, 35), except during periods of inanition or when undergoing ecdysis and pupation. Stress conditions such as these tend to make the gut *p*H of all insects lower, and more uniform along the entire midgut. These changes appear to create conditions in the gut more favorable for bacterial growth and invasion (24). The second group maintains a midgut *p*H below 9.0 but usually above 7.0, except during the above-mentioned stresses or metamorphic stages (65, 66, 55, 13, 14, 24). Other factors excluded, this second group of Lepidoptera is more susceptible to bacterial infection than are insects with a midgut having a high *p*H.

The phytophagous Hymenoptera usually have a much lower midgut *p*H that ranges from 6.5 to 9.0. Bacteria may grow well in the gut of these insects. Significantly, they are susceptible to infection by such organisms as *B. cereus* Frankland and Frankland (25) and *Serratia marcescens* Bizio.

Antibacterial substances in the food. The reaction of the midgut contents is only one of the factors governing the multiplication or existence of bacteria and other microorganisms. The food eaten may contain antimicrobial substances, particularly among the leaf feeders. Nickell (39) reviewed the literature on the study of antimicrobial substances in plants, covering several hundred species from 157 families. Of these, he reported 1,262 species containing antimicrobial agents effective against both gram-negative and gram-positive bacteria, viruses, fungi, and yeasts.

Kushner and Harvey (32) reported the detection of antibacterial substances mainly in forest tree foliage of 17 plant species. They carried out in vitro tests using foliage extract against the insect pathogens *B. cereus*, *B. thuringiensis* var. *thuringiensis*, *B. thuringiensis* var. *sotto* Ishihara, *B. thuringiensis* var. *alesti* Toumanoff and Vago, *Pseudomonas aeruginosa* (Schroeter) Migula, and *S. marcescens*. They found that the extracted plant juices were very effective in preventing growth of *B. cereus* and *B. thuringiensis* varieties and less effective against the nonsporeformers tested. Most interesting was the fact that these antibacterial substances persisted in the gut contents of the insects examined, and that gut juice pressed from dissected insects fed appropriate foliage also strongly inhibited the sporeforming bacteria. These antibacterial substances, at least from the conifers, may fall into the phenolic class of compounds (B. Maksymiuk, *personal communication*).

Antibacterial substances occurring naturally in the insect. Still other antibacterial agents have been investigated in insects. Glaser (21) conducted one of the first series of experiments demonstrating the phenomenon of antibacterial resistance in insects. He injected massive doses of *Coccobacillus acridiorum* (d'Herelle) and *B. poncei* Glaser into the red-legged grasshopper [*Melanoplus femurrubrum* (De Geer)]. There was no excessive phagocytosis; yet the bacteria disappeared from the blood in 25 hr. Glaser felt that this disappearance demonstrated the presence of "extracellular antagonistic substances" in the hemolymph. He made no attempt to separate these substances from the blood.

Eckstein (18) stated that there appeared to be active principles in insects which afford them

"the functions of decontamination or protection against extraneous substances."

Hindle and Duncan (27) found that *B. anthracis* Cohn, *Staphylococcus aureas* Rosenbach, and the typhoid bacillus were killed in vitro by the stomach contents of the fowl tick [*Argas persicus* (Oken)]. They were unable to recover the typhoid bacillus from the feces, but they did find that it could survive in the digestive tract for 22 days. These investigators proposed two alternate explanations for this phenomenon: either some unknown bactericidal principle was at work in the hind portion of the digestive tract or the bacteria were killed by their own metabolites.

This work was followed by Duncan's more comprehensive study of the susceptibility of 18 species of bacteria to the gut contents and feces of 7 species of arthropods (16). Duncan noted that in all tests the hemolymph of the arthropods was ineffective against the bacteria. He showed that the antibacterial principle was in the digestive tract of the insects and arachnids. The greatest destruction of bacteria incubated with gut contents of *Stomoxys* and *Argas* took place in the first 2 hr. Duncan showed that sporeformers and staphylococci were susceptible to the active principle, and *B. subtilis* Cohn proved to be the most sensitive of the 18 species of bacteria tested. This active substance was water-soluble, insoluble in fat solvents, and not harmed by digestion with trypsin. It was also thermostable and not altered by storage in the dried state for periods up to 6 months.

Violle and Sautet (63) reported "bacterial sterilization" powers in pupal and adult forms of the northern house mosquito [*Culex pipiens pipiens* Linnaeus]. The bactericidal principle was the strongest in the pupal stage, but was not present in the larvae collected in the field. Two substances, separable on the basis of tolerance to heat, were described. The organism these investigators used in their studies was called *Bacterium coli*.

According to Olivier (40), an acetone extract of ground larvae of the greater wax moth [*Galleria mellonella* (Linnaeus)] caused lysis of tubercle bacilli (called by the author "Bacilli of Arloing and Courmont"). The acetone extract was washed with 0.1 N NaOH, adjusted to *p*H 7.5, and concentrated. The concentrate was then added to veal broth containing tubercle bacilli from 4-day-old cultures.

Frings, Goldberg, and Grentzen (19) demonstrated a bacterial principle in the blood of the large milkweed bug [*Oncopeltus fasciatus* (Dallas)] that was effective against *S. aureus* and to a lesser degree against *B. subtilis*. Further work on this principle was carried out recently by Gingrich (20). He found that blood from one insect, in vitro, was roughly the equivalent of 1 Oxford unit of pencillin against *S. aureus*. The antibacterial substance is water-soluble, insoluble in ether and ethyl alcohol, and not a protein precipitated by boiling.

In 1950, Simmons et al. (51) reported that exudates from third-stage grubs of the common cattle grub [*Hypoderma lineotum* (de Villers)] and the northern cattle grub [*Hypoderma bovis* (Linnaeus)] showed considerable antibacterial activity in vitro against *Escherichia coli*, *Micrococcus pyogenes* var. *aureus*, and *Salmonella typhosa*, but not against *B. subtilis*. He demonstrated that active material was in the guts of the insect larvae. The antibacterial substance was stable in the refrigerator and withstood boiling for 25 min without loss of activity.

Pavan (43) announced his success in purifying an antibiotic from total body extracts of the Argentine ant [*Iridomyrex humilus* (Mayr)]. This crystalline extract, named Iridomirmecina, is capable of halting growth of a large group of human and other vertebrate pathogens.

Pavan showed that *I. humilus* does not produce formic acid, and none of the ant species that produce formic acid contain Iridomirmecina. Each ant contains approximately 0.003453 mg of this substance during the summer, or about 1% of the body weight. The material, produced in special abdominal glands, is concentrated in a large reservoir in the dorsal tip of the abdomen.

Iridomirmecina is stable at room temperature for several months and remains active after incubation at 120 C for 30 min or at 37 C for 10 days.

Rehm (45) detected an antibacterial substance in the blood of the larval silkworm that inhibited the growth of typhoid bacilli, hemolytic staphylococci, streptococci, penumococci, gonococci, etc. He named this substance Insectin. This substance undoubtedly is the same antibacterial agent described by Briggs (7). However, Rehm quite obviously thought of this material as an antibiotic, whereas Brigg's description tends to eliminate it as such a compound.

Perhaps one of the most interesting and controversial problems involving antibacterial activity in insects was introduced by Baer (2), who reported the remarkable freedom from infections due to staphylococci and streptococci in war wounds infested with maggots. Several years after his first observation, Baer treated four children having chronic osteomyelitis with laboratory-reared larvae of the blow fly *Lucilia phaenici* (= *sericata*) (Meigen). For approximately 5 years, Baer's technique was employed, with both favorable and unfavorable reports appearing in

the literature. Baer felt that there was some substance formed by the activity of the maggots, but he made no attempt to isolate the material. He drew attention to the fact that the wound changed to an alkaline *p*H 24 hr after the maggots were implanted; this change suggested "... some biochemical effect within the wound causing a constitutional reaction inimical to bacterial growth."

Livingston and Prince (34) claimed that a filtered extract of larval brei contained an active principle concentrated enough to completely cure 95 of 100 cases of micrococcal and tubercular infections. The investigators made no attempt to determine what the active principle was or how it acted.

Weil, Simon, and Sweadner (67) concluded that two principles were involved in the healing action of maggots: (i) that the maggots acted as scavengers, cleaning the wound of debris, and (ii) that the larvae excreted proteolytic substances which hastened liquefaction of necrotic tissue, thus cleaning the wound and allowing more rapid recovery.

Slocum, McClellan, and Messer (54) did not obtain any "active principle" from macerated larvae, but attributed the action of the larvae to their ability to change the *p*H of the wound to alkalinity and also to the massaging effect of the larvae moving in the wound. Simmons (52, 53) reported that *L. sericata* excreted a powerful bactericidal agent and claimed that its action accounted for the therapeutic value of maggot treatment.

Gwatkin and Fallis (23) showed that washings from larvae of the blow flies, *Calliphora vicina* (Robineau-Desvoidy) [= *Calliphora erythrocephala* (Meigen)], *Eucalliphora lilaea* (Walker) (= *Calliphora latrifrons* Hough), and other species, contained bactericidal substances effective against *S. aureus*, *Streptococcus agalactiae* (Lehmann and Neumann), two strains of *Brucella abortus* (Schmidt and Weis), and *Salmonella typhosa* (Zoph.) in vitro. They also stated that the bactericidal effect was significant after successive rearings of the fly larvae were carried out in the laboratory.

Further investigation of this much-debated problem is obviously needed, since no specific action attributable to the maggots has yet been proved to the satisfaction of everyone. There is a dearth of reports on this problem in the literature of the past few years.

In summation of this review of insect antimicrobial capacity, it is clear that many insects possess extensive defenses against invasion by, and development of, bacteria in the gut. Indeed, the gut may be the insect's most important line of defense.

HUMORAL AND CELLULAR IMMUNITY

If an infectious agent does manage to invade the hemocoele, the host still has several defense mechanisms available. One or more of these may act to preserve a state of health. Primarily, these factors are humoral immunity and cellular mechanisms.

We will limit our discussion here to the most recent investigations, insofar as possible.

Humoral Responses

The whole subject under discussion in this section was exhaustively reviewed by Briggs (7, 8), Wagner (64), and Stephens (60). Briefly, they concluded that weak nonspecific antibacterial or bactericidal substances may be found in the blood of insects, and that these substances can be quantitatively enhanced by injection of specific or nonspecific antigens (7, 8, 20, 45, 57, 58, 59, 60), as described above. However, all these investigators concluded that antibodies, as they are known, are nonexistent in insect blood. Stephens (60) stated that no globulinlike proteins appear in insect blood, but Bernheimer et al. (4) pointed out that transplantation of organs between individual larvae of the regal moth [*Citheronia regalis* (Fabricius)] and other Lepidoptera was unopposed by any defensive system such as is found in vertebrates. Plagge (44) suggested that there was little evidence of immune response, which implied a lack of antibodies as we know them.

This is rather impressive evidence against the theory that insects produce true antibodies. Eventually, the conclusions drawn by the above reviewers and investigators may be proved correct. However, some phenomena reported in the literature warrant further study. For example, Whittager and West (68) found proteins that migrated cathodically in their starch-gel electrophoresis of insect blood. They even went so far as to state that there was some similarity between γ-globulin and their cathodic fraction.

Again, perusal of the reports of Briggs (7) and Stephens (57) indicated that their work was carried out exclusively with bacteria which are highly virulent for the test insects. Briggs used a strain of *E. coli* that did not kill the silkworm at dosages above 12 million cells per insect for a control organism, and as a vaccine in tests with *P. aeruginosa* (MLD, 120 cells per insect) and *M. pyogenes* (MLD, 1,200 cells per insect). Neither Briggs nor Stephens paused to examine *E. coli* and other bacteria to which their test insects were obviously immune. Granted, the insects are susceptible to

many species of bacteria, but they are also extremely resistant to many other species. The burning question is: what protects the silkworm from *E. coli* and other "nonpathogens"? Whatever the answer, an immune response must be involved.

Recently, Bullock (10), using disc-electrophoresis techniques, showed that melanization caused removal of proteins from the hemolymph. This interesting observation has been expanded by Bullock and by S. R. Dutky (*personal communication*) in the light of past work; in some cases, it might be postulated that melanization is detrimental to insect resistance to bacterial infection. Later we will discuss the role of melanization as a possible protective mechanism.

Stephens (60) pointed out that blood from the greater wax moth melanizes on exposure to air. Certainly melanization takes place when the insect is injected with a virulent *P. aeruginosa* culture. However, Stephens noted that the ability to melanize was not present in insects previously injected with *P. aeruginosa* vaccine. Moreover, these vaccinated insects were more resistant to the pathogen. Briggs (8) pointed out that stored *B. mori* blood slowly melanizes, and the loss of antibacterial activity is proportional to the degree of melanization. Two possible explanations might be proposed to explain these phenomena: (i) the process of melanin formation ties up phenolic compounds free in the blood, and phenols are nonspecific germicides; and (ii) the proteins, bound to melanin, are removed from solution during the process of melanization. The disappearance of the bound proteins may also be correlated with a drop in immune reaction.

To support this line of thought, we should briefly discuss an excellent piece of research by Gingrich (20). This investigator found that agar micro-electrophoresis of cell-free blood from actively immunized *O. fasciatus* produced several protein bands, all of which inhibited *P. aeruginosa* when the bacterium was grown directly on the electrophoresis slide. He showed that the action of the antibacterial substance was lysis of the bacterium. Gingrich separated the proteins with trichloroacetic acid from cell-free blood (immunized insect) and found that the lytic factor was reduced, but not destroyed. He therefore concluded that the lytic factor was a smaller molecule, intimately bound to the serum proteins, but not a protein itself.

Apparently, the recent work by Briggs, Stephens, Bullock, and Gingrich may be related. Some insects do possess antibacterial substances. It is even apparent that the proteins present in the blood may be involved, although indirectly, and there is good reason to suspect that phenolic compounds may be involved in the active systems.

In insects there seems to be little evidence for antibodies similar to those found in vertebrates; however, there is some evidence that insect blood contains substances causing immune responses, but these substances are incompletely understood. Certainly we must investigate why some bacteria, injected into the blood in large numbers, fail to kill the insects.

Cellular Immunity

Hemocytes of insects often actively respond to invading microorganisms, encapsulating the large entities while smaller bodies, such as individual bacterial rods, may be phagocytized in the true sense. Actually, the phenomenon of phagocytosis was first studied in insects. Before the turn of the century, Metchnikoff (38) had already observed that the wandering cells of the water flea *Daphnia* phagocytized the spores of an infectious yeast immediately upon their entrance into the hemolymph through the gut wall, and caused the spores to disintegrate. Occasionally, when a spore escaped immediate engulfment, it would burst into rapidly budding yeast cells which secreted "a poison" that not only repelled the phagocytes but killed them "by dissolving them completely." When this happened, the insect died.

From succeeding studies, we have learned that the bulk of the blood cells participate in this phagocytic function (70, 28), and that these hemocytes respond positively to a great variety of materials. In the excellent experiments of Salt (49, 50), blood cells of the tomato moth *Diataraxia oleracea* Linnaeus responded to practically everything foreign or unnatural to the insect. For example, wounds, alien parasites, and all kinds of organic and inorganic inert materials, such as silk and cotton threads, glass rods, rose and cactus thorns, and celloidin globules, were encapsulated. Also invested by blood cells were many interspecific and most intraspecific tissue transplants, the response being weakest to transplants from closely related individuals. There was no blood-cell response to its own healthy tissue, intraspecific tissue transplants, or to parasites habitually infesting this moth.

As described by Salt (49), the blood cells of *Diataraxia* similarly encapsulate all foreign particles animate or inanimate. At 6 to 10 hr after a foreign entity is introduced, the blood cells have accumulated in a covering 60 to 100 μ thick. At 24 hr, the capsule is still about 100 μ in thickness but, except on the periphery, the blood cells have become flattened and densely packed. By the 2nd day, there are two distinct layers, an inner, relatively clear layer, usually 30 to 50 μ thick, and

an outer layer in which the laminar structure of the flattened cells remains evident.

When the hymenopterous parasite, *Nemeritus*, is encapsulated, it usually dies within 24 hr, most likely from asphyxiation. After 4 days, it has shrivelled up to a size no larger than the head capsule. By the 11th day, many of the blood cells have withdrawn, and the capsule persists unmolested indefinitely.

Salt (46, 47, 48, 49, 50) found that the only major difference between the formation of capsules around inert objects and around living material was that melanin was deposited on the latter. He felt that these deposits were the result of tyrosinase, which oxidizes blood phenols to melanin and is released from blood cells damaged mechanically or chemically by the material being invested. Although these deposits may contribute to the death of unnatural parasites, Salt (48) stated that, " . . . it is a matter of opinion whether it should be considered a defense reaction or merely a reaction that sometimes has a protective effect."

The inner transparent layer of the capsule is considered to be mucopolysaccharide. Wigglesworth (69, 70) showed that the most numerous type of hemocyte in *Rhodnius*, "the phagocytic amoebocyte," contains periodic acid-Schiff-positive inclusions which are discharged over the inner surface of the basement membrane at the formation of new cuticle during the molting period. Connective tissue membranes around fat body, muscle fibers, and other internal structures, foreign bodies included, appeared to be formed in a similar fashion.

Salt (49, 50) suggested that the phagocytes are able to recognize bodies foreign or unnatural to the insect by the lack of this mucopolysaccharide surface. During encapsulation, this surface is provided by the blood cells, many of which then withdraw. His well-supported theory does not explain why a capsule contains several layers of hemocytes when only the first layer has contact with the inadequate surface. Since biologically inert materials are invested, any chemotaxis must be among the phagocytes themselves. It also follows that contact between hemocytes and extraneous material is by chance, insofar as they are funneled into close proximity as the blood percolates through the viscera. More information in this area would be desirable.

In spite of the facts presented here, the hemocytes are incapable of preventing septicemia and death from all microorganisms. In fact, Cameron (11) concluded that the phagocytes of caterpillars were very passive toward some bacteria but were unable to kill, or at least contain, others. We still have no explanation for this phenomenon, but bacterial compounds toxic to the hemocytes are probably often released. Bucher (9) pointed out that some pathogenic bacteria " . . . produce strong proteolytic enzymes . . . " which, he suggested, " . . . may be responsible for degenerative changes in the phagocytic cells of the host and for the digestion of the host tissues . . . . " The possibility of blood cells being lysed certainly warrants direct investigation.

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